Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzyme activities in different tissues of *Sarotherodon mossambicus* (Peters) exposed to a carbamate pesticide, carbaryl

Murugesan Ramaswamy,[1]* Palaniswamy Thangavel[2] and N Panneer Selvam[1]

[1]*Department of Zoology, Government Arts College (Autonomous), Coimbatore - 641 018, Tamil Nadu, India*
[2]*Department of Environmental Sciences, Bharathiar University, Coimbatore - 641 046, Tamil Nadu, India*

Abstract: The activity levels of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzymes were estimated in liver, muscle and brain tissues of the fish, *Sarotherodon mossambicus* (Peters), which had been exposed to sub-lethal (3 mg litre^{-1}) and lethal (25 mg litre^{-1}) concentrations of the carbamate insecticide carbaryl. Based on the results obtained, the changes in GOT and GPT levels in liver, muscle and brain following different periods of sub-lethal and lethal carbaryl exposures suggested that *S mossambicus* showed adaptive elevation in the activity levels of the two aminotransferase enzymes in the tissues, thereby probably aiding gluconeogenesis through transamination of glucogenic amino acids to meet the energy demand under carbaryl toxicity.

Keywords: carbaryl; *Sarotherodon mossambicus*; toxicity; gluconeogenesis

1 INTRODUCTION

Pesticides are known to affect different physiological parameters of fish such as oxygen consumption,[1,2] haematological parameters[2–4] and carbohydrate metabolism.[1,5,6] A shift in carbohydrate metabolic pathway towards anaerobic breakdown of glycogen has been reported in fish.[1] Though glutamic oxaloacetic transaminase (GOT, L-aspartate 2-oxoglutarate aminotransferase, EC 2.6.1.1) and glutamic pyruvic transaminase (GPT, L-alanine 2-oxoglutarate aminotransferase, EC 2.6.1.2) enzymes are known to play a key role in mobilizing L-amino acids for gluconeogenesis and function as links between carbohydrate and protein metabolism under altered physiological, pathological and induced environmental stress conditions,[7–9] studies on the effects of pesticides on these aminotransferases of fish tissues are sparse.[10–17]

The carbamate pesticide, carbaryl (sevin; 1-naphthyl *N*-methylcarbamate) was selected for the present study. It is effective against pests of cotton, red palm weevil on coconut and pests of vegetable crops,[18] and is a very stable insecticide retaining its full potency over a prolonged period in the environment after use.[19] Carbaryl is a common and widely used pesticide for cotton pests in Coimbatore district.

In view of this, the present project studies the effects of carbaryl on the levels of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities of different tissues of a freshwater, edible fish, *Sarotherodon mossambicus* (Peters) (Class: Osteichthyes; Order: Actinopterygii).

2 MATERIALS AND METHODS

2.1 Fish exposure tests

Sarotherodon mossambicus ranging in weight from 10 to 12 g, collected from lakes in and around Coimbatore city, were kept in large cement tanks for acclimatization for one month and were fed regularly with boiled egg and cooked rice. One week before the start of the investigation, a randomly selected batch of healthy fish was transferred to a small cement cistern and maintained under laboratory conditions (29 ($\pm$1) °C). Feeding was discontinued one day before the fish were used in the experiment.

A commercially available carbaryl 100 g kg^{-1} dust (Rhone-Poulenc Agrochemicals India Ltd; Batch Number 170) was used in the present study. Chlorine-free tap water was used to prepare the required concentrations of pesticide suspension to which fish

* Correspondence to: Murugesan Ramaswamy, Department of Zoology, Government Arts College (Autonomous), Coimbatore - 641 018, Tamil Nadu, India
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were exposed. In the present study, fish were exposed to potentially sub-lethal (3 mg litre^{-1}) and potentially lethal (25 mg litre^{-1}) concentrations of carbaryl, based on the toxicity studies of Venkateswaran and Ramaswamy.[20]

Twenty litres of pesticide water containing either a sub-lethal (3 mg litre^{-1}) or a lethal (25 mg litre^{-1}) concentration of carbaryl was taken in a 50-litre glass jar. Ten fish (at the rate of 2 litres per fish) were introduced into each glass jar containing the pesticide + water and the jar covered with wire mesh. Control fish were maintained in similar glass jars containing pesticide-free tap water for a maximum period of 15 days. The pesticide + water was renewed every 12 h (under sub-lethal exposure) in order to maintain the dissolved oxygen content and pesticide concentration in the jar. Fish were not fed during the experimental exposure period.

Six surviving fish from each jar were killed (by a blow on the head) after 5, 10 and 15 days in the case of control and sub-lethal treatments. In the case of lethal dose of carbaryl, fish were killed at 1, 2 and 3 h of exposure, since a sufficient number of swimming fish were not available after 3 h.

2.2 Preparation of tissue extract

Samples of tissues from liver, muscle and brain were dissected out from each fish (maintained in an ice-tray). Weighed tissue samples from liver, muscle and brain were used for the estimation of activity of GOT and GPT enzymes by the method of Reitman and Frankel[21] as follows:

A 5% homogenate was prepared in phosphate buffer (0.1 M, pH 7.4) at 0 °C and centrifuged at 3000 rev min^{-1} for 15 min. The supernatant was collected and used in the estimation of GOT and GPT enzyme activity levels.

2.3 Estimation of GOT and GPT activities

Buffered aspartate-α-ketoglutarate substrate (0.5 ml; pH 7.4) (for GOT) and buffered alanine-α-ketoglutarate substrate (0.5 ml; pH 7.4) (for GPT) were incubated in two test tubes separately at 37 °C for 5 min. Tissue extract (0.1 ml) was added to each of the incubated buffered substrates, mixed well and incubated at 37 °C for 60 min. After 60 min incubation, 0.5 ml of 2,4-dinitrophenyl hydrazine colour reagent was added to each of the incubated mixtures, mixed well and allowed to stand at room temperature (29 (± 1) °C) for 20 min. After 20 min, 5 ml of 4 M sodium hydroxide solution was added to each of the incubated mixtures, mixed well and allowed to stand at room temperature for 10 min. The optical density (OD) of the colour developed in the two incubated mixtures was read separately at 505 nm (using distilled water as blank) in a photoelectric colorimeter (Systronics, model-112).

Standard graphs were constructed for known activity levels of GOT enzyme by using buffered aspartate substrate and 2 mM pyruvate standard solution and for known enzyme activity levels of GPT enzyme by using buffered alanine substrate and 2 mM pyruvate standard solution (supplied by Ranbaxy Laboratories Ltd, New Delhi, India). The activity levels of GOT and GPT enzymes in liver, muscle and brain of control and carbaryl exposed *S mossambicus* were obtained from the standard graphs and expressed as units per milligram of tissue. The changes in the activity levels of GOT and GPT enzymes in liver, muscle and brain (either increase or decrease) of carbaryl exposed *S mossambicus* from those of control levels were calculated as percentages.

2.4 Statistical analysis

Throughout this study, six individual estimations were done for each tissue under each period of exposure and the average was taken for discussion. The differences in the mean values of GOT and GPT enzyme activity levels in liver, muscle and brain of control and carbaryl exposed (for 5, 10 and 15 days under sub-lethal exposure and for 1, 2 and 3 h under lethal exposure) fish were tested for their significance using Student's '*t*' test.[22] Data were also analysed statistically by Analysis of Variance (ANOVA or '*F*') test using one-way classification and by Duncan's Multiple Range Test (DMRT).[23]

3 RESULTS

Data on the GOT and GPT enzyme activity levels in liver, muscle and brain tissues of control, potentially sub-lethal and potentially lethal carbaryl-exposed fish for different periods are given in Tables 1 and 2.

3.1 Glutamic oxaloacetic transaminase (GOT) activity levels in carbaryl-exposed fish

Exposure of *S mossambicus* to a sub-lethal concentration (3 mg litre^{-1}) of carbaryl caused a significant elevation in GOT levels during the initial period of exposure in all the three tissues. However, prolonged exposure for 10 days and 15 days elicited lesser elevation in the GOT levels of the tissues. Among the tissues studied, maximum elevation of GOT enzyme level (+296 %) was observed in liver during the initial period of sub-lethal exposure.

Compared to the sub-lethal exposure, lethal exposure (to 25 mg litre^{-1} of carbaryl) caused higher magnitudes of elevation during the initial period of 1 h in all three tissues. Prolonged lethal exposure for 2 and 3 h caused lesser but significant elevation in the GOT levels of the three tissues (Table 1).

3.2 Glutamic pyruvic transaminase (GPT) activity levels in carbaryl-exposed fish

The activity levels of GPT enzymes also showed elevated levels following different periods of sub-lethal and lethal exposures of the fish to carbaryl. Like GOT, GPT level also showed a large elevation in the liver tissue compared to that of muscle and brain tissues. However, under sub-lethal exposure, GPT levels

Table 1. Glutamic oxaloacetic transaminase (GOT) activity levels in liver, muscle and brain tissues of control, lethal (25 mg litre^{-1}) and sub-lethal (3 mg litre^{-1}) carbaryl-exposed *Sarotherodon mossambicus*

		GOT activity level (units mg^{-1} tissue)^{a,b,c} ($\pm$SE)^{d} [change from control (%)]							
		Potentially lethal exposure (h)				Potentially sub-lethal exposure (days)			
Tissue		1	2	3	F value	5	10	15	F value
Liver	Control	1.63 (±0.02)a	1.64 (±0.05)a	1.58 (±0.01)a	1.02	1.62 (±0.09)a	1.62 (±0.07)a	1.61 (±0.08)a	1.14
	Exposed	10.00 (±1.26)**a [+515]	3.47 (±0.12)**c [+111]	3.84 (±0.09)**b [+142]	12.48	6.43 (±0.93)**a [+296]	3.27 (±0.05)**a [+103]	1.82 (±0.06)†d [+13]	8.48
Muscle	Control	1.64 (±0.05)a	1.61 (±0.03)a	1.60 (±0.03)a	1.68	1.65 (±0.12)a	1.61 (±0.09)a	1.59 (±0.11)a	1.26
	Exposed	9.98 (±1.46)**a [+508]	3.46 (±0.04)**c [+115]	3.60 (±0.03)**b [+126]	7.26	5.01 (±0.47)**a [+203]	3.53 (±2.24)**b [+119]	1.83 (±0.08)†d [+15]	9.21
Brain	Control	2.34 (±0.27)a	2.32 (±0.22)a	2.32 (±0.19)a	0.92	2.45 (±0.31)a	2.30 (±0.29)a	2.23 (±0.33)a	1.04
	Exposed	10.06 (±1.29)**a [+330]	3.97 (±0.02)**c [+71]	5.23 (±0.01)**b [+126]	10.42	5.83 (±0.37)**a [+138]	3.64 (±0.19)**b [+58]	3.05 (±0.20)*c [+37]	12.46

[a] ** Significant difference from control, $P < 0.01$; * Significant difference from control, $P < 0.05$; † No significant difference from control, $P > 0.05$.
[b] $F(0.05) = 2.74$; $F(0.01) = 4.14$.
[c] In a row, mean values followed by a common letter are not significantly different at the 5% level by DMRT.
[d] $n = 6$.

progressively increased up to 10 days of exposure in all three tissues estimated. Prolonged exposure for 15 days caused lesser and insignificant (+ 15 %) elevation in the liver tissue together with lesser but significant elevation in muscle (+46 %) and brain (+55 %) tissues.

Lethal exposure of *S mossambicus* elicited higher elevation in all three tissues, with maximum elevation (+402 %) in the liver tissue. However, unlike sub-lethal exposure, GPT levels registered lesser but significant elevations following 2 and 3 h of lethal exposures (Table 2).

4 DISCUSSION

Transaminases are enzymes involved in the catabolism of amino acids and are involved in the catalytic process of interconversion of a pair of amino acids and a pair of α-keto acids. The enzymes GOT and GPT are known to act as a link between carbohydrate and protein metabolism.

Exposure of fish to pesticides showed changes in the levels of GOT and GPT enzyme in different tissues. Lane and Scura[13] reported increased transaminase activity levels in different tissues of the sailfin molly, *Poscillia latipinna* exposed to a low concentration (0.003 mg litre^{-1}) of dieldrin. Elevation in aspartate aminotransferase (AAT $\equiv$ GOT) and alanine aminotransferase (AlAT $\equiv$ GPT) activity levels of different tissues was also reported in *Anabas testudineus* (Block) exposed to lethal (10.5 mg litre^{-1}) and sub-lethal (4 mg litre^{-1}) concentration of disyston.[11] Bakthavathsalam and Srinivasa Reddy,[10] while studying the intoxication effects of lindane on the activities of AAT and AlAT in different tissues of *A testudineus*, also reported an elevation in the enzymes in different tissues studied. Narasimha Murthy *et al*[12] also reported that the levels of AAT and AlAT enzymes increased in brain, liver, muscle and gill of *Oreochromis*

Table 2. Glutamic pyruvic transaminase (GPT) activity levels in liver, muscle and brain tissues of control, lethal (25 mg litre^{-1}) and sub-lethal (3 mg litre^{-1}) carbaryl-exposed *Sarotherodon mossambicus*

		GPT activity level (units mg^{-1} tissue)^{a,b,c} ($\pm$SE)^{d} [change from control (%)]							
		Potentially lethal exposure (h)				Potentially sub-lethal exposure (days)			
Tissue		1	2	3	F value	5	10	15	F value
Liver	Control	2.23 (±0.04)*a	2.10 (±0.05)*a	2.00 (±0.02)*a	1.49	2.23 (±0.14)*a	2.11 (±0.09)*a	1.99 (±0.10)*a	1.62
	Exposed	11.18 (±1.16)**a [+402]	3.27 (±0.05)**c [+55]	4.00 (±0.06)**b [+101]	8.42	5.54 (±0.12)**a [+149]	5.71 (±0.55)**b [+170]	2.29 (±0.03)†d [+15]	9.46
Muscle	Control	1.71 (±0.06)*a	1.67 (±0.04)*a	1.65 (±0.05)*a	1.17	1.86 (±0.16)a	1.59 (±0.12)*a	1.58 (±0.08)*a	1.73
	Exposed	6.76 (±0.39)**a [+296]	2.56 (±0.05)**c [+53]	3.13 (±0.05)**b [+90]	11.36	4.02 (±0.16)**a [+117]	5.60 (±0.67)**b [+252]	2.31 (±0.08)**d [+46]	13.14
Brain	Control	2.47 (±1.98)*a	2.42 (±1.89)*a	2.39 (±1.63)*a	0.87	2.48 (±1.78)*a	2.42 (±1.82)*a	2.39 (±1.56)*a	1.06
	Exposed	6.03 (±0.30)**a [+144]	3.26 (±0.04)**c [+35]	4.79 (±0.14)**b [+100]	9.25	4.11 (±0.12)**a [+66]	4.55 (±0.76)**b [+88]	3.68 (±0.11)**c [+55]	10.95

[a] ** Significant difference from control, $P < 0.01$; * Significant difference from control, $P < 0.05$; † No significant difference from control, $P > 0.05$.
[b] $F(0.05) = 2.74$; $F(0.01) = 4.14$.
[c] In a row, mean values followed by a common letter are not significantly different at the 5% level by DMRT.
[d] $n = 6$.

mossambicus exposed to lethal (0.15 mg litre^{-1}) and sub-lethal (0.05 mg litre^{-1}) concentrations of lindane. Elevated levels of GOT and GPT enzymes were reported in different tissues of *Tilapia mossambica* exposed to parathion-methyl;[14] in *Channa striatus* exposed to malathion and phosphamidon;[15] in rosy barb, *Puntius conchonius* exposed to aldicarb, phosphamidon and endosulfan[16] and in *Cyprinus carpio* exposed to paraquat.[17]

In the present study, exposure of *S mossambicus* to potentially sub-lethal (3 mg litre^{-1}) and potentially lethal (25 mg litre^{-1}) concentrations of carbaryl caused unequivocal elevation in the levels of GOT and GPT enzymes in liver, muscle and brain. The large increase in the activity levels of GOT and GPT (ranging from 140 to 518%), particularly in liver and muscle, following five days of sub-lethal exposure or 1 h of lethal exposure (Tables 1 and 2) clearly indicates that fish adaptively increase the activity levels of tissue GOT and GPT to meet the energy demand under pesticide stress, possibly by promoting gluconeogenesis. GOT and GPT are known to play a strategic role in mobilizing L-amino acids (like alanine and aspartic acid) for gluconeogenesis.[7,8] The lesser magnitude of increase in GOT and GPT levels in brain tissue (except the level of GOT under lethal exposure) compared to liver and muscle, probably indicates the lesser metabolic importance of brain tissue.

Following 10 days of sub-lethal exposure or under 2 h of lethal exposure, levels of GOT and GPT showed a declining trend in liver and muscle tissues. The declining trend in the aminotransferases levels was well pronounced following prolonged exposure for 10 days under sub-lethal exposure or after 3 h under lethal exposure (showing either very low or insignificant elevation in the levels). This declining trend in the GOT and GPT levels of the three tissues following prolonged exposure could possibly be due to the histopathological changes caused in the tissues following prolonged pesticide exposure. Sarojini and Ramaswamy[24] reported severe pathological lesions induced by carbaryl in the liver of *Tilapia mossambica* upon prolonged exposure and also to higher concentration of the pesticide.

A study of Tables 1 and 2 also indicates that the magnitude of adaptive elevation of GOT and GPT levels is greater (ranging from 330% to 515%) during the early period (1 h) of lethal exposure than during the early period (five days) of sub-lethal exposure. This could be taken to suggest that, under lethal concentrations, the fish suffers a severe stress thereby adaptively increasing its tissue GOT and GPT levels to meet the energy demand (by aiding gluconeogenesis) under lethal carbaryl toxicity.

The large increase in GOT and GPT levels following initial periods of exposure followed by a drop in the same levels and a further elevation (particularly under lethal exposure) during prolonged exposure, observed uniformly in all the three tissues, is indicative of the reversible action of the carbamate pesticide, carbaryl. The latter has been reported to reversibly inhibit tissue acetylcholinesterase activity,[25] thereby attesting to its neurotoxic mode of action in the fish tissue[18] and acid and alkaline phosphatase activities.[26] The observed restoration of near control levels of GOT enzyme, particularly in liver tissue during prolonged exposure for 15 days, could be taken to suggest that the histopathological changes caused by sub-lethal concentrations of carbaryl are less and reversible, indicating the selective adaptive responses of the fish tissue under sub-lethal concentrations.

In conclusion, it could be stated that *S mossambicus*, when exposed to sub-lethal and lethal concentrations of carbaryl, showed adaptive elevation in the activity levels of GOT and GPT enzymes, particularly in liver and muscle, thereby probably aiding gluconeogenesis through transamination of glucogenic amino acids to meet the energy demand under carbaryl toxicity.

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